

L Number	Hits	Search Text	DB	Time stamp
1	371	HS-40	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/04 17:58
3	60	HS-40 and promoter	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/04 17:59
2	14	HS-40 and Zeta\$15	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/04 18:00
4	61	HS-40 and (promoter OR enhancer)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/04 17:59
5	4	(US-6303845-\$).did. or (US-20020148000-\$ or US-20020133838-\$ or US-20020108134-\$).did.	USPAT; US-PGPUB	2004/08/04 17:59
6	2	("5270184").PN.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/04 17:59
9	2	("6022738").PN.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/04 17:59
8	6	TCTGAGTCA	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/04 17:59
10	8	(HS-40 OR Zeta NEAR globin).clm.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/04 18:02
11	408	HS-40 OR Zeta NEAR globin	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/04 18:02
12	26	(HS-40 OR Zeta NEAR globin) and retrovir\$5	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/04 18:02
13	7	(US-6303845-\$ or US-6022738-\$ or US-6524851-\$).did. or (US-20020108134-\$ or US-20020148000-\$ or US-20020133838-\$).did. or (US-20020133838-\$).did.	USPAT; US-PGPUB; DERWENT	2004/08/04 18:04

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(FILE 'HOME' ENTERED AT 17:48:28 ON 04 AUG 2004)

FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, MEDICONF' ENTERED  
AT 17:48:47 ON 04 AUG 2004

L1 871 S ZETA (L) GLOBIN  
L2 0 S HS-40 (L) ENAHNCER  
L3 326 S HS-40  
L4 0 S S1 (L) L3  
L5 58 S L1 (L) L3  
L6 25 DUP REM L5 (33 DUPLICATES REMOVED)  
L7 15 S L6 AND PY<=1998  
L8 15 SORT L7 PY  
L9 10 S L6 NOT L7  
L10 10 SORT L9 PY

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L10 ANSWER 2 OF 10 MEDLINE on STN  
AN 2000153760 MEDLINE  
TI Loading of DNA-binding factors to an erythroid enhancer.  
SO Molecular and cellular biology, (2000 Mar) 20 (6) 1993-2003.  
Journal code: 8109087. ISSN: 0270-7306.  
AU Wen S C; Roder K; Hu K Y; Rombel I; Gavva N R; Daftari P; Kuo Y Y; Wang C;  
Shen C K  
AB The **HS-40** enhancer is the major cis-acting regulatory  
element responsible for the developmental stage- and erythroid  
lineage-specific expression of the human alpha-like **globin**  
genes, the embryonic **zeta** and the adult alpha2/alpha1. A model  
has been proposed in which competitive factor binding at one of the  
**HS-40** motifs, 3'-NA, modulates the capability of  
**HS-40** to activate the embryonic **zeta**-  
**globin** promoter. Furthermore, this modulation was thought to be  
mediated through configurational changes of the **HS-40**  
enhanceosome during development. In this study, we have further  
investigated the molecular basis of this model. First, human erythroid  
K562 cells stably integrated with various **HS-40**  
mutants cis linked to a human alpha-**globin** promoter-growth  
hormone hybrid gene were analyzed by genomic footprinting and expression  
analysis. By the assay, we demonstrate that factors bound at different  
motifs of **HS-40** indeed act in concert to build a fully  
functional enhanceosome. Thus, modification of factor binding at a single  
motif could drastically change the configuration and function of the  
**HS-40** enhanceosome. Second, a specific 1-bp, GC-->TA  
mutation in the 3'-NA motif of **HS-40**, 3'-NA(II), has  
been shown previously to cause significant derepression of the embryonic  
**zeta-globin** promoter activity in erythroid cells. This  
derepression was hypothesized to be regulated through competitive binding  
of different nuclear factors, in particular AP1 and NF-E2, to the 3'-NA  
motif. By gel mobility shift and transient cotransfection assays, we now  
show that 3'-NA(II) mutation completely abolishes the binding of small  
MafK homodimer. Surprisingly, NF-E2 as well as AP1 can still bind to the  
3'-NA(II) sequence. The association constants of both NF-E2 and AP1 are  
similar to their interactions with the wild-type 3'-NA motif. However,  
the 3'-NA(II) mutation causes an approximately twofold reduction of the  
binding affinity of NF-E2 factor to the 3'-NA motif. This reduction of  
affinity could be accounted for by a twofold-higher rate of dissociation  
of the NF-E2-3'-NA(II) complex. Finally, we show by chromatin  
immunoprecipitation experiments that only binding of NF-E2, not AP1, could  
be detected in vivo in K562 cells around the **HS-40**  
region. These data exclude a role for AP1 in the developmental regulation  
of the human alpha-**globin** locus via the 3'-NA motif of  
**HS-40** in embryonic/fetal erythroid cells. Furthermore,  
extrapolation of the in vitro binding studies suggests that factors other  
than NF-E2, such as the small Maf homodimers, are likely involved in the  
regulation of the **HS-40** function in vivo.

L10 ANSWER 5 OF 10 CAPLUS COPYRIGHT 2004 ACS on STN

AN 2001:757858 CAPLUS  
 DN 135:314417  
 TI Vectors containing mutated **HS-40** enhancer of .  
**zeta.-globin** gene promoter and its regulation of  
 transgene expression in transgenic mice  
 SO U.S., 7 pp., Division of U.S. Ser. No. 205,015, abandoned.  
 CODEN: USXXAM  
 IN Shen, Che-Kun James  
 AB The invention relates to a mutated **HS-40** enhancer of .  
**zeta.-globin** gene promoter, a 350-400 bp enhancer  
 element located about 40 kb upstream of **.zeta.-globin**  
 gene. **HS-40** is the major cis-acting regulatory  
 element responsible for the developmental stage-and erythroid  
 lineage-specific expression of the human  $\alpha$ -like **globin**  
 genes, the embryonic **.zeta.** and the adult  $\alpha 2/\alpha 1$ .  
 The invention is based on the discovery that a single nucleotide change in  
 the 3'NF-E2/AP1 element of the human **HS-40** enhancer,  
 unlike the wild type **HS-40** enhancer, confers  
 position-independent and copy number-dependent expression on a transgene. In  
 addition, the single nucleotide change allows expression of the gene in the  
 cells of an adult mouse, an effect not seen for the wild type **HS**  
**-40** enhancer. Accordingly, the invention provides a viral  
 expression vector (e.g., a retrovirus) expressing a transgene regulated by  
 (1) a transcriptional start site; (2) a promoter (e.g., a tissue-specific  
 promoter such as **.zeta.-globin** promoter) operably  
 linked to the transcriptional start site; and (3) the above mutated  
**HS-40** enhancer operably linked to the promoter.

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 6303845	B1	20011016	US 2000-536094	20000324
	US 2002133838	A1	20020919	US 2001-961563	20010920
	US 2002108134	A1	20020808	US 2001-977432	20011015
	US 2002148000	A1	20021010	US 2001-14220	20011109

L10 ANSWER 7 OF 10 CAPLUS COPYRIGHT 2004 ACS on STN

AN 2003:1004738 CAPLUS

DN 140:1576

TI A strong variant of the **HS-40** enhancer and its use in expression vectors  
 for transgenic animals

SO U.S. Pat. Appl. Publ., 13 pp., Cont.-in-part of U.S. Ser. No. 961,563.

CODEN: USXXCO

IN Shen, Che-kun James

AB A substitution mutant of the **HS-40** enhancer of .  
**zeta.-globin** gene promoter, a 350-400 bp enhancer  
 element located about 40 kb upstream of **.zeta.-globin**  
 gene is used in expression vectors for high level expression of foreign  
 genes in transgenic animals. **HS-40** is the major  
 cis-acting regulatory element responsible for the developmental stage-and  
 erythroid lineage-specific expression of the human  $\alpha$ -like  
**globin** genes, the embryonic **.zeta.** and the adult  
 $\alpha 2/\alpha 1$ . A single nucleotide change in the 3'NF-E2/AP1 element  
 of the human **HS-40** enhancer, unlike the wild type  
**HS-40** enhancer, confers position-independent and copy  
 number-dependent expression on a transgene. The mutation also relieves the  
 developmental regulation of expression from the promoter of the .  
**zeta.-globin** gene. In addition, the single nucleotide  
 change allows expression of the gene in the cells of an adult mouse, an  
 effect not seen for the wild type **HS-40** enhancer. The  
 transgenic animal may include pig, rat, cow, rabbit, goat, guinea pig,  
 prairie baboon, squirrel, monkey, chimpanzee, bird, frog, toad, chicken,  
 turkey and sheep. The generation of transgenic mice expressing a growth  
 hormone gene in erythroblasts using the **HS-40(mt)**  
 enhancer and the **.zeta.-globin** promoter is  
 demonstrated. Serum growth hormone levels of up to 6,490 ng/mL were  
 obtained.

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2002148000	A1	20021010	US 2001-14220	20011109
	US 6303845	B1	20011016	US 2000-536094	20000324

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L7 15 S L6 AND PY<=1998  
L8 15 SORT L7 PY

=> d an ti so au ab pi l8 1 5 7 9 10

L8 ANSWER 1 OF 15 MEDLINE on STN  
AN 91342671 MEDLINE  
TI Characterization of the major regulatory element upstream of the human  
alpha-globin gene cluster.  
SO Molecular and cellular biology, (1991 Sep) 11 (9) 4679-89.  
Journal code: 8109087. ISSN: 0270-7306.  
AU Jarman A P; Wood W G; Sharpe J A; Gourdon G; Ayyub H; Higgs D R  
AB The major positive regulatory activity of the human alpha-globin  
gene complex has been localized to an element associated with a strong  
erythroid-specific DNase I hypersensitive site (HS -40  
) located 40 kb upstream of the zeta 2-globin mRNA cap  
site. Footprint and gel shift analyses of the element have demonstrated  
the presence of four binding sites for the nuclear factor GATA-1 and two  
sites corresponding to the AP-1 consensus binding sequence. This region  
resembles one of the major elements of the beta-globin locus  
control region in its constitution and characteristics; this together with  
evidence from expression studies suggests that HS -40  
is a primary element controlling alpha-globin gene expression.

L8 ANSWER 5 OF 15 MEDLINE on STN  
AN 93204975 MEDLINE  
TI Transcriptional activation of human zeta 2 globin  
promoter by the alpha globin regulatory element (HS-  
40): functional role of specific nuclear factor-DNA complexes.  
SO Molecular and cellular biology, (1993 Apr) 13 (4) 2298-308.  
Journal code: 8109087. ISSN: 0270-7306.  
AU Zhang Q; Reddy P M; Yu C Y; Bastiani C; Higgs D; Stamatoyannopoulos G;  
Papayannopoulou T; Shen C K  
AB We studied the functional interaction between human embryonic zeta  
2 globin promoter and the alpha globin regulatory  
element (HS-40) located 40 kb upstream of the  
zeta 2 globin gene. It was shown by transient  
expression assay that HS-40 behaved as an authentic  
enhancer for high-level zeta 2 globin promoter  
activity in K562 cells, an erythroid cell line of embryonic and/or fetal  
origin. Although sequences located between -559 and -88 of the  
zeta 2 globin gene were dispensable for its expression  
on enhancerless plasmids, they were required for the HS-  
40 enhancer-mediated activity of the zeta 2  
globin promoter. Site-directed mutagenesis demonstrated that this  
HS-40 enhancer-zeta 2 globin  
promoter interaction is mediated by the two GATA-1 factor binding motifs  
located at -230 and -104, respectively. The functional domains of  
HS-40 were also mapped. Bal 31 deletion mapping data  
suggested that one GATA-1 motif, one GT motif, and two NF-E2/AP1 motifs  
together formed the functional core of HS-40 in the  
erythroid-specific activation of the zeta 2 globin  
promoter. Site-directed mutagenesis further demonstrated that the  
enhancer function of one of the two NF-E2/AP1 motifs of HS-  
40 is mediated through its binding to NF-E2 but not AP1  
transcription factor. Finally, we did genomic footprinting of the  
HS-40 enhancer region in K562 cells, adult nucleated

erythroblasts, and different nonerythroid cells. All sequence motifs within the functional core of **HS-40**, as mapped by transient expression analysis, appeared to bind a nuclear factor(s) in living K562 cells but not in nonerythroid cells. On the other hand, only one of the apparently nonfunctional sequence motifs was bound with factors in vivo. In comparison to K562, nucleated erythroblasts from adult human bone marrow exhibited a similar but nonidentical pattern of nuclear factor binding in vivo at the **HS-40** region. These data suggest that transcriptional activation of human embryonic **zeta 2 globin** gene and the fetal/adult alpha **globin** genes is mediated by erythroid cell-specific and developmental stage-specific nuclear factor-DNA complexes which form at the enhancer (**HS-40**) and the **globin** promoters.

L8 ANSWER 7 OF 15 MEDLINE on STN  
 AN 95327665 MEDLINE  
 TI Transcriptional activation of human adult alpha-globin genes by hypersensitive site-40 enhancer: function of nuclear factor-binding motifs occupied in erythroid cells.  
 SO Proceedings of the National Academy of Sciences of the United States of America, (1995 Jul 3) 92 (14) 6454-8.  
 Journal code: 7505876. ISSN: 0027-8424.  
 AU Rombel I; Hu K Y; Zhang Q; Papayannopoulou T; Stamatoyannopoulos G; Shen C K  
 AB The developmental stage- and erythroid lineage-specific activation of the human embryonic **zeta**- and fetal/adult alpha-**globin** genes is controlled by an upstream regulatory element [hypersensitive site (**HS**)-**40**] with locus control region properties, a process mediated by multiple nuclear factor-DNA complexes. In vitro DNase I protection experiments of the two G+C-rich, adult alpha-**globin** promoters have revealed a number of binding sites for nuclear factors that are common to HeLa and K-562 extracts. However, genomic footprinting analysis has demonstrated that only a subset of these sites, clustered between -130 and +1, is occupied in an erythroid tissue-specific manner. The function of these in vivo-occupied motifs of the alpha-**globin** promoters, as well as those previously mapped in the **HS-40** region, is assayed by site-directed mutagenesis and transient expression in embryonic/fetal erythroid K-562 cells. These studies, together with our expression data on the human embryonic **zeta-globin** promoter, provide a comprehensive view of the functional roles of individual nuclear factor-DNA complexes in the final stages of transcriptional activation of the human alpha-like **globin** promoters by the **HS-40** element.

L8 ANSWER 9 OF 15 MEDLINE on STN  
 AN 95238333 MEDLINE  
 TI Functional roles of in vivo footprinted DNA motifs within an alpha-globin enhancer. Erythroid lineage and developmental stage specificities.  
 SO Journal of biological chemistry, (1995 Apr 14) 270 (15) 8501-5.  
 Journal code: 2985121R. ISSN: 0021-9258.  
 AU Zhang Q; Rombel I; Reddy G N; Gang J B; Shen C K  
 AB Transcriptional regulation of the human alpha-like **globin** genes, embryonic **zeta 2** and adult alpha, during erythroid development is mediated by a distal enhancer, **HS-40**. Previous protein-DNA binding studies have shown that **HS-40** consists of multiple nuclear factor binding motifs that are occupied in vivo in an erythroid lineage- and developmental stage-specific manner. We have systematically analyzed the functional roles of these factor binding motifs of **HS-40** by site-directed mutagenesis and transient expression assay in erythroid cell cultures. Three of these **HS-40** enhancer motifs, 5'NF-E2/AP1, GT II, and GATA-1(c), positively regulate the **zeta 2-globin** promoter activity in embryonic/fetal erythroid K562 cells and the adult alpha-**globin** promoter activity in adult erythroid MEL cells. On the other hand, the 3'NF-E2/AP1 motif is able to exert both positive and negative regulatory effects on the **zeta 2-globin** promoter activity in K562 cells, and this dual function appears to be modulated through differential binding of the ubiquitous AP1 factors and the erythroid-enriched NF-E2 factor. Mutation in the GATA-1(d) motif,

which exhibits an adult erythroid-specific genomic footprint, decreases the **HS-40** enhancer function in dimethyl sulfoxide-induced MEL cells but not in K562 cells. These studies have defined the regulatory roles of the different **HS-40** motifs. The remarkable correlation between genomic footprinting data and the mutagenesis results also suggests that the erythroid lineage- and developmental stage-specific regulation of human alpha-like **globin** promoters is indeed modulated by stable binding of specific nuclear factors in vivo.

L8 ANSWER 10 OF 15 CAPLUS COPYRIGHT 2004 ACS on STN  
AN 1996:128940 CAPLUS  
DN 124:166363  
TI Transcriptional regulation of human  $\zeta 2$  and  $\alpha$  globin promoters  
by multiple nuclear factor-DNA complexes: The final act  
SO Molecular Biology of Hemoglobin Switching, Proceedings of the Conference  
on Hemoglobin Switching, 9th, Orcas Island, Wash., June 10-14, 1994 (1995), Meeting Date 1994, 193-202. Editor(s): Stamatoyannopoulos, George. Publisher: Intercept, Andover, UK.  
CODEN: 62JIAN  
AU Zhang, Qingyi; Rombel, Irene; Reddy, G. Narender; Shen, C. -K. James  
AB A review, with 29 refs. Site-directed mutagenesis and transient expression assay were used to analyze functional contributions of individual nuclear factor-binding motifs to the transcriptional regulation of the two human  $\alpha$ -like **globin** promoters, embryonic **zeta**.2, and adult  $\alpha$ , by the **HS-40** element in embryonic/fetal erythroid K562 cells and adult erythroid MEL cells.

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